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(54) Title: **ESTRIOL THERAPY FOR MULTIPLE SCLEROSIS AND OTHER AUTOIMMUNE DISEASES**

(57) Abstract: The present invention discloses administering steroid hormones to mammals to treat autoimmune related diseases, more particularly, Th1-mediated (cell-mediated) autoimmune diseases including: multiple sclerosis (MS), rheumatoid arthritis (RA), autoimmune thyroiditis and uveitis. Most preferably the invention is used to treat a patient with a therapeutically effective amount of estriol of 8 milligrams once daily via oral administration to treat the symptoms or prevent the onset of multiple sclerosis.

ESTRIOL THERAPY FOR MULTIPLE SCLEROSIS AND OTHER AUTOIMMUNE DISEASES

BACKGROUND OF THE INVENTION

[0001] 1. *Field of the Invention.* This invention relates generally to steroidal therapies for treating autoimmune diseases and, more particularly, to administering primary agents being estrogens or estrogen receptor active agents for the treatment of cell mediated diseases. Optionally, secondary agents which effect the immune system may also be co-administered. Finally, treatment kits are provided containing at least one primary agent and at least one secondary agent for treating a patient presenting with symptomology of an autoimmune disease.

[0002] 2. *General Background and State of the Art.* There is a distinct female preponderance of autoimmune diseases during the reproductive ages including multiple sclerosis (MS), rheumatoid arthritis (RA), uveitis, myasthenia gravis (MG), Sjogren's syndrome, and Hashimoto's thyroiditis.

[0003] For example, MS is a chronic, and often debilitating disease affecting the central nervous system (brain and spinal cord). MS affects more than 1 million people worldwide and is the most common neurological disease among young adults, particularly woman. The exact cause of MS is still unknown. MS attacks the nervous system resulting in myelin sheaths surrounding neuronal axons to be destroyed. This demyelination can cause weakness, impaired vision, loss of balance, and poor muscle coordination. MS can have different patterns, sometimes leaving patients relatively well after episodes of acute worsening, sometimes leading to progressive disability that persists after episodes of worsening. In the worst cases the disease can lead to paralysis or blindness.

[0004] Steroid hormones or sex-linked gene inheritance may be responsible for the enhanced susceptibility of women to these autoimmune diseases. A role for steroid hormones in susceptibility to autoimmune disease is supported by observations of alternations in disease symptomatology, with alterations in sex hormone levels such as during pregnancy, menopause or exogenous hormone administration (in the form of

hormone replacement (HRT) or oral contraceptives (ORC)). For example, women with MS and RA have been reported to experience remission of symptoms during late gestation. Particularly, MS patients have been reported to show a decrease in relapse rate in pregnancy.

[0005] Normally, cell-mediated immunity is mediated by T helper cell (Th1) secretion of interferon gamma (IFN- γ) and tumor necrosis factor beta (TNF-b). In contrast, humoral immunity is mediated by another group of T helper cells (Th2) secreting interleukin (IL)-10, IL-4, IL-5 and IL-6. A systemic shift toward humoral immunity (or Th2-mediated immunity) has been noted during pregnancy. During pregnancy, cell-mediated immunity is decreased and humoral-mediated immunity is increased thereby promoting fetal survival. Thus, this systemic shift in the immune system may explain why cell-mediated diseases, including MS and RA have been reported to improve during pregnancy.

[0006] Although a shift toward humoral-mediated immunity has been demonstrated during human pregnancy, mechanisms which induce this shift remain unclear. One possibility is local production of Th2 (or humoral mediated) cytokines by the placenta. Another possibility is the production of Th2 cytokines by immune cells, consequent to changed levels of steroid hormones during pregnancy. Consistent with the latter possibility, *in vitro* studies have demonstrated the ability of the steroid progesterone to increase IL-4 production and the ability of the steroid 17 β -estradiol to increase IL-10 production during T-lymphocyte responses. However, it remains unclear what cellular mechanisms are involved in regulating *in vivo* amelioration of autoimmune symptomology.

[0007] Examples of potential candidates which effect may effect MS during pregnancy include: Sex hormones (estrogens, progesterone), cortisol, vitamin D, alpha-fetoprotein, human chorionic gonadotropin and pregnancy specific glycoproteins.

[0008] Further, some studies have suggested that a unique pregnancy factor termed "early pregnancy factor" is responsible for improved progression of cell-mediated autoimmune diseases during pregnancy. Other studies have suggested a role for microchimerism. Still others suggest a role for local factors such as TGF β or estriol (E3)

which is known to be produced by the placenta during pregnancy. Of note, E3 is at its highest serum levels in the third trimester of pregnancy. However, E3's role in ameliorating symptoms of autoimmune diseases in humans is unclear.

[0009] Studies in laboratory animals have established that experimental autoimmune encephalomyelitis (EAE) and other Th1 (cell-mediated) autoimmune diseases in mice improve during pregnancy.

[0010] Specifically, treatment with late pregnancy levels of estriol or supraphysiological doses of estradiol (5 times pregnancy levels) were shown to delay the onset of clinical EAE after disease was experimentally induced by immunization of mice (Jansson et al. 1994). However, there was no investigation as to how estrogens delayed the day of onset of disease, nor as to whether disease severity was effected in these animals once symptomology occurred.

[0011] In another study, it was shown that EAE disease severity could be reduced by treatment with estriol, either before or after disease onset. Treatment of EAE mice with 90 day release pellets of 5 milligrams or 15 milligrams of estriol (E3) was shown not only to decrease disease severity but also to enhance autoantigen specific humoral-immunity, increase production of the Th2 cytokine IL-10 and reduced inflammation and demyelination in EAE mice. Importantly, these changes in the disease were induced by a dose (5mg) which was shown to yield estriol levels in serum that were similar to those which occur during late pregnancy (Kim et al., *Neurology*, 50(4 Supp. 4):A242-245, April 1998, *FASEB Journal* 12(4):A616, March 1998 and *Neurology* 52(6):1230-1238, April 1999; herein incorporated by reference). Thus, these results suggested that steroid hormones, and estriol in particular, may be involved in the amelioration of autoimmune reactions in the EAE animal model.

[0012] Other groups later demonstrated that estrogen potentiated the effects of treatment with TCR proteins to reduce autoimmune reactions in EAE mice. Offner, et al. *FASEB Journal* 14(6):A1246, April 2000; *Int. Journal of Mol. Medicine* 6 (Supp. 1): S8, October 2000 and *Journal of Clin. Invest.* 105(10):1465-1472, May 2000). Further, it was shown in animal studies that estrogen suppressed the onset EAE in mice (Ito, et al. *Journal of Immunology*, 167(1): 452-52, 2001) and that presumed diestrus levels of

estrogens reduced some manifestations of active EAE in mice. Bebo et al. *Journal of Immunology* 166(3): 2080-9, 2001.

[0013] However, the etiology and disease progression of EAE and MS are not identical, thus it is unclear that estrogens alone would be effective in ameliorating autoimmune responses in human patients. Indeed, not only is it unknown whether pregnancy doses of estrogens might be protective in humans with autoimmune disease, it is unclear even in mice whether low doses of estrogens are protective. For example, it has been reported by some that ovariectomy of female mice makes EAE disease worse (Matejuk et al., 2001), while others have found that ovariectomy had no effect on disease severity (Kim et al., 2001; Voskuhl and Palaszynski, 2001a; Voskuhl and Palaszynski, 2001b). Thus, it is controversial whether low levels of estrogens, as they exist during the menstrual cycle, are protective even in mice.

[0014] Data from human studies to date have shown no clear benefit of steroids in treating any autoimmune disease. In humans, administration of available hormone therapies (including HRTs and OCPs) containing a mixture of sex hormones cause some autoimmune diseases to improve while others worsen.

[0015] For example, there has been no conclusive evidence that women are protected from or have a decrease in symptomology or relapse rates due to sex steroids. One study noted that past use of oral contraceptives in healthy women had no effect on subsequent risk to develop MS (Hernan et al. 2000). Further, another study found that the incidence rates for MS in current users were not decreased as compared to never-users (Thorogood and Hannaford, 1998). Thus, low dose of the estrogens in oral contraceptives are not of sufficient type or dose to ameliorate the immunopathogenesis of MS even temporarily during intercurrent use. At best, in one study, patients had the subjective impression that pre-existing MS symptoms (as opposed to relapse rates) worsen during the premenstrual period and that the use of oral contraceptives may have decreased this worsening (Zorgdrager and De Keyser, 1997). Importantly, the lack of reports of an effect of oral contraceptive therapy on MS relapses is in marked contrast to what has been observed during pregnancy.

[0016] In contrast, it has been shown that women had a lower the risk of developing MS during pregnancy compared to non-pregnant states (Runmarker and Andersen, 1995). Due to the numerous changes that occur during pregnancy, hormonal and nonhormonal (as listed above), the etiology of the beneficial effect of pregnancy may or may be related to sex steroid fluctuations. It has also been reported for decades that pregnancy decreases MS relapses (Abramsky, 1994; Birk et al. 1990; Birk et al, 1998; Damek and Shuster, 1997; Runmarker and Andersen, 1995; Confavreux et al., 1998). These studies have shown that the latter part of pregnancy is associated with a significant reduction in relapses, while there is a rebound increase in relapses post partum. In contrast, the absence of such an effect on relapses during OCP or HRT indicate that low level sex steroids are not adequate to treat these symptoms.

[0017] Further, women having rheumatoid arthritis that were treated with HRT did not show significant improvement in their symptomology. DaSilva and Hall, *Baillieres Clinical Rheumatology* 1992, 6:196-219; Bijlsma et al. *Journal of Repro. Imm.* 28(3-4):231-4, 1992; Hall et al. *Annals of the Rheumatic Diseases*, 53(2): 112-6, 1994.

[0018] Thus, the low doses of hormones found naturally during the menstrual cycle or in ORT and HRT have not been shown to be effective at ameliorating the symptomology of autoimmune diseases. This is in spite of the observation that women having MS have a decreased relapse rate during late pregnancy. Thus, a challenge has been to identify a hormone and a treatment dose that is therapeutic in treating particular autoimmune diseases, while minimizing undesirable side effects. Obviously, the dose and method of administration of steroids in humans differs from steroid treatment in laboratory animals due to toxic effects of prolonged exposure by patients to steroid hormones. In particular, there are clinical concerns of inducing breast or endometrial cancers in women requiring long term exposure to steroid hormones.

INVENTION SUMMARY

[0019] A general object of the present invention is to provide a method of administering steroid hormones to mammals to treat autoimmune related diseases, more particularly, Th1-mediated (cell-mediated) autoimmune diseases including: multiple sclerosis (MS), rheumatoid arthritis (RA), autoimmune thyroiditis, uveitis and other autoimmune

diseases in which clinical symptomology has shown improvement during the third term of pregnancy.

[0020] In accordance with one aspect of the present invention, these objectives are accomplished by providing a treatment for autoimmune related diseases with a selected dose and course of a primary agent being an estrogen or estrogen receptor-effective composition.

[0021] In accordance with one aspect of the present invention, these objectives are accomplished by providing a patient with a therapeutically effective amount of estriol, comprising from about 4 to 16 milligrams per day, or more specifically, about 8 milligrams once daily via oral administration.

[0022] In accordance with another aspect of the present invention, these objectives are accomplished by providing a therapeutically effective amount of a primary agent in combination with a therapeutically effective amount of a secondary active agent, such as progesterone, glucocorticoids and/or known or experimental drugs used to treat autoimmune diseases.

[0023] The above described and many other features and attendant advantages of the present invention will become apparent from a consideration of the following detailed description when considered in conjunction with the accompanying drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

[0024] Fig. 1a is a schematic depicting the trial design described in Example 1; Fig. 1b is a bar graph depicting human serum levels during pregnancy, estriol treatment (Tx), and pretreatment (Pre Tx levels).

[0025] Fig. 2a is a bar graph describing the Delayed Type Hypersensitivity (DTH) responses to tetanus and to candida; Fig. 2b is a bar graph depicting levels of IFN γ between treatment groups.

[0026] Fig. 3a-f are bar graphs depicting each patient's gadolinium enhancing lesion volumes on serial cerebral MRIs which were assessed at each month during the pretreatment, estriol treatment and post treatment periods.

[0027] Fig. 4 is a bar graph depicting mean percent change in PASAT scores during treatment with estriol as compared to pretreatment.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

[0028] This description is not to be taken in a limiting sense, but is made merely for the purpose of illustrating the general principles of the invention. The section titles and overall organization of the present detailed description are for the purpose of convenience only and are not intended to limit the present invention.

[0029] Generally, the invention involves a method of treating mammal exhibiting clinical symptoms of an autoimmune disease comprising administering a primary agent at a therapeutically effective dosage in an effective dosage form at a selected interval. The treatment is aimed at reducing the symptomology and/or progression of the disease. In the preferred embodiment of the invention, human patients clinically diagnosed with MS (including both relapsing remitting or secondary progressive type patients) are treated with an oral preparation of 8 milligrams estriol daily and have ameliorated symptomology.

[0030] Amelioration of the autoimmune disease refers to any observable beneficial effect of the treatment. The beneficial effect can be evidenced by a delayed onset or progression of disease symptomology, a reduction in the severity of some or all of the clinical symptoms, or an improvement in the overall health.

[0031] For example, patients who have clinical symptoms of an autoimmune disease often suffer from some or all of the following symptoms: worsening of pre-existing symptoms (such as joint pain in rheumatoid arthritis), the appearance of new symptoms (new joints affected in rheumatoid arthritis) or increased generalized weakness and fatigue. MS patients in particular suffer from the following symptoms: weakness, numbness, tingling, loss of vision, memory difficulty and extreme fatigue. Thus an amelioration of disease in MS would include a reduction in the frequency or severity of onset of weakness, numbness, tingling, loss of vision, memory difficulty and extreme fatigue. On imaging of the brain (MRI) amelioration of disease would be evidenced by a decrease in the number or volume of gadolinium enhancing lesions, a stabilization or slowing of the accumulation of T2 lesions and/or a slowing in the rate of atrophy.

formation. Immunologically, an increase in Th2 cytokines (such as IL-10) and a decrease in Th1 cytokines (such as interferon gamma) would be associated with disease amelioration.

[0032] Patients may also express criteria indicating they are at risk for developing autoimmune diseases. These patients may be preventatively treated to delay the onset of clinical symptomology. More specifically, patients who present initially with clinically isolated syndromes (CIS) may be treated using the treatment paradigm outlined in this invention. These patients have had at least one clinical event consistent with MS, but have not met full criteria for MS diagnosis since the definite diagnosis requires more than one clinical event at another time (McDonald et al., 2001). Treatment of the present invention would be advantageous at least in preventing or delaying the development of clinically definite MS.

[0033] PRIMARY AGENT. The primary agent useful in this invention is a steroid hormone, more particularly a estrogen or a steroidal or non-steroidal estrogen receptor active agent. Most preferably the primary agent is estriol (estra-1,3,5(10)-triene-3,16,17-triol, E3, such as estriol succinate, estriol dihexanate or estriol sulfmate. However, the primary agent may be precursors or analogs of estriol (such as nyestriol), estrone (E1) or precursors or analogs of estrone, 17 β -estradiol (E2) or precursors (including aromatizable testosterone) or analogs of 17 β -estradiol.

[0034] The primary agent may also be a metabolite or derivatives of E1, E2 or E3 which are active at the estrogen receptor α or β . Metabolites and derivatives may have a similar core structure to E1, E2 or E3 but may have one or more different groups (ex. hydroxyl, ketone, halide, etc.) at one or more ring positions. Synthetic steroids which are effective at estrogen receptor are also useful in this invention, such as those described in WO 97/08188 or US 6043236 to Brattsand.

[0035] The primary agent may also be an estrogen receptor α or β agonists and/or antagonist. These agonists or antagonists may be steroidal or non-steroidal agents which bind to and/or cause a change in activity or binding of at least one of the estrogen receptor α or β subtypes. For example, specific agonists of ER alpha and ER beta may be useful in this invention (Fritzmeier, et al.). Doses of these agonists may be titrated to

achieve an effect on disease similar to that which is observed during pregnancy and during treatment with pregnancy doses of estriol by methodologies known to those skilled in the art of steroid pharmacology.

[0036] Any one or combination of these estrogens or estrogen receptor active agents may be used to treat the selected autoimmune disease. The selection of the estrogens or estrogen receptor active agents can be made considering secondary side effects of the treatment to the patient. For example, estriol may be selected over 17β -estradiol, because estriol causes minimal endometrial proliferation and is not associated with increased risk of breast cancer. Minimal endometrial proliferation is observed when the long-acting estriol derivative, nyestriol is used. Indeed, because estriol has partial antagonist action on the binding of 17β -estradiol to the estrogen receptor *in vivo*, estriol was at one point in the past considered as a therapeutic agent for treatment and prevention of breast cancer.

[0037] THERAPEUTICALLY EFFECTIVE DOSAGE OF THE PRIMARY AGENT. A therapeutically effective dose of the primary agent is one sufficient to raise the serum concentration above basal levels, and preferably to pregnancy levels or above pregnancy levels. Most preferably, the therapeutically effective dosage of the primary agent is selected to result in serum levels in a patient equivalent to the steroid hormone level of that agent in women in the second or third trimester of pregnancy.

[0038] For example, during the normal female menstrual cycle estradiol levels are in the range of about 350 pg/ml serum. During pregnancy, there is about a 100 fold increase in the level of estradiol to about 10,000 to about 35,000 pg/ml serum. Correale, et al. *Journal of Immunology* 161:3365 (1998) and Gilmore, et al. *Journal of Immunology* 158:446. In contrast, estriol levels are undetectable during the menstrual cycle in the non-pregnant state. Estradiol levels rise progressively during pregnancy to levels from 3,000 to 30,000 pg/ml (3 to 30 ng/ml) (www.il-st-acad-sci.org/steroid1.html#se3t).

[0039] In one embodiment, where the primary agent is estriol, the preferable dose is from about 4 to 16 milligrams daily, and more specifically, about 8 milligrams daily. In this embodiment, blood serum levels preferably reach at least about 2 ng/ml, may reach about 10 to about 35 ng/ml, or most preferably about 20-30 ng/ml. Sicotte et al.

Neurology 56:A75. In some embodiments, estradiol (E2) levels would preferably reach at least about 2 ng/ml and most preferably about 10-35 ng/ml. In some embodiments, estrone (E1) levels would preferably reach at least about 2 ng/ml and most preferably about 5-18 ng/ml (DeGroot and Jameson, 1994).

[0040] The dosage of the primary agent may be selected for an individual patient depending upon the route of administration, severity of disease, age and weight of the patient, other medications the patient is taking and other factors normally considered by the attending physician, when determining the individual regimen and dosage level as the most appropriate for a particular patient.

[0041] The use of this group of primary agents is advantageous in at least that other known or experimental treatments for cellular mediated autoimmune diseases are chemotherapeutic immunosuppressants which have significant risks and side effects to patients, including decreasing the ability of the patient to fight infections, inducing liver or heart toxicity which are not caused by estrogen treatment. Other agents used in MS do not cause these side effect, but are associated with flu-like symptoms or chest tightness. Further, these previously used agents are associated with local skin reactions since they entail injections at frequencies ranging from daily to once per week.

[0042] **DOSAGE FORM.** The therapeutically effective dose of the primary agent included in the dosage form is selected at least by considering the type of primary agent selected and the mode of administration. The dosage form may include the active primary agent in combination with other inert ingredients, including adjuvants and pharmaceutically acceptable carriers for the facilitation of dosage to the patient as known to those skilled in the pharmaceutical arts. The dosage form may be any form suitable to cause the primary agent to enter into the tissues of the patient.

[0043] In one embodiment, the dosage form of the primary agent is an oral preparation (liquid, tablet, capsule, caplet or the like) which when consumed results in elevated serum estrogen levels. The oral preparation may comprise conventional carriers including diluents, binders, time release agents, lubricants and disintegrants.

[0044] In other embodiments of the invention, the dosage form may be provided in a topical preparation (lotion, creme ointment or the like) for transdermal application.

Alternatively, the dosage form may be provided in a suppository or the like for transvaginal or transrectal application.

[0045] That estrogens or estrogen receptor active agents can be delivered via these dosage forms is advantageous in that currently available therapies, for MS for example, are all injectables which are inconvenient for the user and lead to decreased patient compliance with the treatment. Non-injectable dosage forms are further advantageous over current injectable treatments which often cause side effects in patients including flu-like symptoms (particularly, β interferon) and injection site reactions which may lead to lipotrophy (particularly, glatiramer acetate copolymer-1).

[0046] However, in additional embodiments, the dosage form may also allow for preparations to be applied subcutaneously, intravenously, intramuscularly or via the respiratory system.

[0047] **SECONDARY ACTIVE AGENTS.** Any one or a combination of secondary active agents may be included in the dosage form with the primary agent. Alternatively, any one or a combination of secondary active agents may be administered independently of the primary agent, but concurrent in time such that the patient is exposed to at least two agents for the treatment of their immunological disease.

[0048] The secondary agents are preferably immunotherapeutic agents, which act synergistically with the primary agent to diminish the symptomology of the autoimmune disease. Secondary active agents may be selected to enhance the effect of the estrogen or estrogen receptor active agent, reduce the effect of the estrogen or estrogen receptor active agent or effect a different system than that effected by the estrogen or estrogen receptor active agent.

[0049] Secondary active agents include immunotherapeutic agents which cause a change in the activity or function of the immune system.

[0050] In one embodiment, a secondary agent may be a therapeutically effective amount of progesterone, precursor, analog or progesterone receptor agonist or antagonist. Most preferably, the secondary agent is 100-200 milligrams of progesterone administered daily. Progesterone in combination with estrogen or estrogen receptor

active agent treatment is advantageous in at least protecting patients against risks associated with long term estrogen exposure, including, but not limited to endometrial proliferation and breast cancers.

[0051] In another embodiment, a secondary agent may be a therapeutically effective amount of glucocorticoid, precursor, analog or glucocorticoid receptor agonist or antagonist. For example, prednisone may be administered, most preferably in the dosage range of about 5-60 milligrams per day. Also, methyl prednisone (Solumedrol) may be administered, most preferably in the dosage range of about 1-2 milligrams per day. Glucocorticoids are currently used to treat relapse episodes in MS patients, and symptomatic RA within this dosage range.

[0052] In other embodiments, a secondary agent may be selected from the group immunotherapeutic compounds. For example, as β -interferon (Avonex® (interferon-beta 1a), Rebiff® (by Serono); Biogen, Betaseron® (interferon-beta 1b) Berlex, Schering), glatiramer acetate copolymer-1 (Copaxone®; Teva), antineoplastics (such as mitoxantrone; Novatrone® Lederle Labs), human monoclonal antibodies (such as natalizumab; Antegren® Elan Corp. and Biogen Inc.), immunosuppressants (such as mycophenolate mofetil; CellCept® Hoffman-LaRoche Inc.), paclitaxel (Taxol®; Bristol-Meyers Oncology), cyclosporine (such as cyclosporin A), corticosteroids (glucocorticoids, such as prednisone and methyl prednisone), azathioprine, cyclophosphamide, methotrexate, cladribine, 4-aminopyridine and tizanidine.

[0053] By way of example, which is consistent with the current therapeutic uses for these treatments, Avonex® in a dosage of about 0 to about 30 mcg may be injected intramuscularly once a week. Betaseron® in a dosage of about 0 to about 0.25 mg may be injected subcutaneously every other day. Copaxone® in a dosage of about 0 to about 20 mg may be injected subcutaneously every day. Finally, Rebiff® may be injected at a therapeutic dose and at an interval to be determined based on clinical trial data. However, dosages and method of administration may be altered to maximize the effect of these therapies in conjunction with estrogen treatment. Dosages may be altered using criteria that are known to those skilled in the art of diagnosing and treating autoimmune diseases.

[0054] Preferably, secondary agents would be administered in the dosage ranges currently used to treat patients having autoimmune diseases, including MS patients. Alternatively, the secondary agents may be administered at a reduced dose or with reduced frequency due to synergistic or duplicative physiological effects with the primary agent.

[0055] Preferably, patients exhibiting symptomology of autoimmune diseases are treated with the above agents (estrogen or estrogen receptor active agents with or without secondary agents). Most preferably, patients exhibit autoimmune diseases marked by improvement in symptomology at least during a treatment regimen, including but not limited to that reflecting patterns observed during the second or third trimester of pregnancy.

[0056] **KITS.** In another aspect of this invention kits are provided for use by the treating physician in the clinic or prescribed patient for self-administration of treatment. The kits of this invention include at least one primary agent and one secondary agent in the appropriate dosages and dosage form for the treatment of the patient's clinical symptoms.

[0057] In a first embodiment of the kit, the primary agent is estriol in doses of about 4-16 milligrams and the secondary agent is progesterone in doses of about 100 to about 200 milligrams. In a second embodiment of this kit, the primary agent is estriol in doses of about 4-16 milligrams and the secondary agent is a glucocorticoid, such as prednisone (about 5-60 milligrams per day) or methyl prednisone (1-2 milligrams per day).

[0058] In a third embodiment of this invention, the primary agent is estriol in doses of about 4-16 milligrams and the secondary agent is β -interferon in doses of about 0.25 milligrams of Betaseron® or 30mcg of Avonex®. In a fourth alternate embodiment of the kit, the primary agent is estriol in doses of about 4 to about 16 milligrams and the secondary agent is glatiramer acetate copolymer in doses of about 20 milligrams of Copaxone®.

[0059] The kit also preferably contains instructions for use of the kit by the use by the treating physician or patients to treat their autoimmune disease. Such information

would include at least the schedule for the administration of the primary agent dose and the secondary agent dose.

[0060] Although the present invention has been described in terms of the preferred embodiment above, numerous modifications and/or additions to the above-described preferred embodiments would be readily apparent to one skilled in the art.

[0061] EXAMPLE 1

[0062] Methods: Trial Design. A crossover design was used with monthly brain MRIs during the six month pretreatment period, the six month treatment period with oral estriol (8milligrams/day) and the six month post treatment period, with clinical and laboratory evaluations as demonstrated (Fig 1A).

[0063] Inclusion Criteria. Women with clinically definite MS, ages 18–50, with an EDSS 0–6.5 who had been off interferon beta and copolymer-1 for at least six months, and had no steroid treatment for at least three months were eligible. At least 5 cm³ of lesion burden on a screening T2 weighted brain MRI was required. Subjects who were pregnant or nursing, on oral contraceptives or hormone replacement therapy, or who had a history of thrombosis, neoplasm or gynecologic disease, or who had been treated in the past with total lymphoid irradiation, monoclonal antibody, T cell vaccination, cladribine or bone marrow transplantation were excluded.

[0064] Patients. Twelve female patients with clinically definite MS were enrolled. Six had RR disease and six had SP disease. All six RR and four of six SP patients completed the entire 18 month study period. One SP patient was discontinued from the study because of prolonged treatment with steroids for tonic spasms by an outside neurologist and the other did not wish to go untreated in the post treatment period. Of the ten patients who completed the entire study, the mean age was 44 years (range 28 to 50 years) and the mean EDSS was 3.3 (range 1.0 to 6.5). The mean EDSS score for the SP patients was 5.0 while the mean EDSS for the RR patients was 2.2. The 18 month trial was extended in RR patients whereby treatment was re-instituted.

[0065] Medication. For the initial treatment phase, micronized, U.S.P. graded estriol powder (Medisca, Inc., Plattsburg, NY) was put into capsules by UCLA Pharmaceutical

Services. During the extension re-treatment phase in the RR patients, all but one received a capsule of estriol (8milligrams/day) plus progesterone (100milligrams/day), while the single RR patient who had a hysterectomy received only estriol (8milligrams/day) (Women's International Pharmacy, Madison, WI).

[0066] *Clinical and Safety Measures.* Subjects were evaluated using the Kurtzke's Expanded Disability Status Scale (EDSS) by the same neurologist (RV) throughout the study. At each visit the study nurse (RK) administered the paced auditory serial addition test (PASAT) and the 9-hole peg test. Blood was drawn for SMA12, cholesterol panel, blood counts and hormone levels (estriol, estradiol, estrone, LH, FSH, cortisol, progesterone). Estriol levels in serum were determined by ELISA according to manufacturer's instructions (Oxford Biomedical, Oxford, Michigan).

[0067] *Delayed Type Hypersensitivity Responses (DTH).* DTH to tetanus (Tetanus Toxoid, Wyeth Laboratories, Marietta, PA) and candida (Candin, Allarmed Laboratories, San Diego, CA) were tested at two timepoints, once in the pretreatment period at study month 3 and once at the end of the treatment period at study month 12 (Figure 1a). A group of six untreated healthy control women were also tested twice, spanning the same time interval (9 months). 0.1 ml of each solution was injected intradermally on the anterior surface of the forearm. Induration at each injection site was read after 48 hours. Each site was measured twice, once vertically and once horizontally with the average recorded. The same nurse (RK) administered all injections and read all responses on all subjects at both time points.

[0068] *Reverse Transcription and Polymerase Chain Reaction.* Peripheral blood mononuclear cells (PBMCs) were isolated from heparinized venous blood and cryopreserved. PBMCs were thawed in parallel from a given patient during the two pre-treatment timepoints and the two treatment timepoints. Total RNA was isolated, DNA was removed and mRNA was reverse transcribed. Both IFN- γ and actin were amplified from the same cDNA, however, the cDNA was diluted 1:9 prior to amplification for actin. Amplification was done in 1 mM MilligramsCl₂ using IFN γ and actin primer sequences (Life Technologies, Rockville, MD). Complementary DNA was amplified for 35 cycles:

45" @ 95°C, 60" @ 54°C and 45" @ 72°C. PCR products were separated on a 1.5% agarose gel containing ethidium bromide and densitometry performed.

[0069] *MRIs.* Scans were performed on a 1.5T G.E. scanner. The pulse sequences obtained were a T1-weighted scan with and without gadolinium (Omniscan 0.1mmol/kg) and a PD/T2 weighted scan. Digitized image data was transferred to a SGI workstation (Silicon Graphics, Inc) for further processing. The number and volume of new and total gadolinium enhancing lesions was determined using a semiautomated threshold based technique (Display, Montreal Neurological Institute) by a single experienced operator (NS). The operator was blinded as to whether patients had RR or SP disease. To calculate T2 volumes, a custom semiautomated, threshold based, seed-growing algorithm was used to determine lesion volume after skull stripping, rf correction and spatial normalization. All scans were counted by the same technician who was blinded as to whether patients had RR or SP disease.

[0070] *Statistical Analysis.* One sample, paired, t tests were used to ascertain significance of percent changes in DTH responses, IFN γ levels and PASAT cognitive testing scores during treatment as compared to pretreatment. The nonparametric, Wilcoxon's signed rank test was used for statistical comparisons in enhancing lesion numbers and volumes on MRI between the six month baseline period and each treatment period, post treatment period and re-treatment period.

[0071] *Results. Estriol levels and tolerability.* Serum estriol levels during treatment and re-treatment approximated those observed in women who were six months pregnant, but were lower than those who were 8.5 months pregnant (Fig 1B). Consistent with previous reports, estriol was well tolerated with only menstrual cycle abnormalities. There were no significant alterations in any laboratory measures including LH, FSH, cortisol, progesterone, estradiol and estrone.

[0072] *Immune Responses.* Skin testing to tetanus and candida were performed once in the pretreatment period and once at the end of the treatment period to determine whether they might be decreased with treatment. DTH responses to tetanus were significantly, $P = 0.006$, decreased at study month 12, when patients had been on estriol for six months, as compared to DTH responses at study month 3, the pretreatment

baseline (Fig 2A). DTH responses to candida were decreased less dramatically and more variably. The significant decrease in DTH responses to tetanus from pretreatment (month 3) to treatment (month 12) was not merely due to repeat testing at nine months since healthy, untreated female controls tested at baseline, then again after nine months, did not demonstrate a significant decrease in DTH responses as compared to their baseline. These findings are consistent with an estriol induced down-regulation of Th1 responses *in vivo* during treatment.

[0073] IFN γ is a signature cytokine for Th1 responses. Therefore, we assessed IFN γ levels by RT-PCR of unstimulated peripheral blood mononuclear cells (PBMCs) derived *ex vivo* from patients during the pretreatment and the treatment periods. In the six RR patients, levels of IFN γ were variably decreased at study month 9 (after three months of estriol treatment) and then significantly decreased, $P = 0.003$, at study month 12 (after six months of estriol treatment) as compared to baseline pretreatment levels (months 3 and 6) (Fig 2B). In contrast, there was no decrease in IFN γ in the four SP patients. These data are consistent with the concept that the immune system of RR patients, as compared to SP patients, may be more amenable to treatments that aim to decrease Th1 responses. Also, the observation that estriol treatment can alter cytokine production by PMBCs is consistent with reports demonstrating estrogen receptors α and β in immune tissues and cells.

[0074] *MRIs*. Based on the protective effect of pregnancy on relapse rates in MS patients and the association of gadolinium enhancing lesions with relapses, we hypothesized that estriol treatment would have an anti-inflammatory effect as manifested by decreases in enhancing lesions on serial brain MRIs. Compared to the six month pretreatment baseline period, the total volume and number of enhancing lesions for all ten MS patients (6RR, 4SP) decreased during the treatment period. This improvement in the group as a whole was driven by the beneficial effect of estriol treatment in the RR, not the SP, group (Fig 3A and 3B). Therapeutic effects of estriol treatment in the RR group were therefore examined in further detail. Within the first three months of treatment of RR patients, median total enhancing lesion volumes were decreased by 79%, $P = 0.02$, and numbers were decreased by 82%, $P = 0.09$ (Fig 3C

and 3D). They remained decreased during the next three months of treatment, with lesion volumes decreased by 82%, $P = 0.01$, and numbers decreased by 82%, $P = 0.02$. In the post treatment period, median total enhancing lesion volumes and numbers became variable in the first three months off treatment, before returning to near baseline levels in the last three months of the post treatment period. During the four month re-treatment extension phase, enhancing lesion volumes decreased again by 88%, $P = 0.008$, and numbers decreased again, this time by 48%, $P = 0.04$, as compared to original baseline (Fig 3C and 3D). Changes in median new enhancing lesion volumes and numbers followed similar patterns as median total lesion numbers and volumes (Fig 3E and 3F).

[0075] Median T2 lesion volumes for the whole group were 15.3 cm^3 (range 6.1 – 33.8), with no significant differences in median T2 volumes between RR and SP groups. Consistent with enhancing lesion data, serial T2 lesion volumes revealed that estriol treatment tended to be most beneficial in RR patients. In the RR group, median T2 lesion volumes remained stable during the six month treatment period (0% change), increased during the six month post treatment period (7.4% higher), and then declined in the four month re-treatment extension period (2.0% lower).

[0076] *Clinical measures.* Relapses were few and showed no significant changes during the study. In the six RR patients, one relapse occurred during the pretreatment period, one in the treatment period, two in the post treatment period and none in the re-treatment period. No relapses occurred in SP patients. EDSS and 9 Hole Peg Test scores showed no significant changes during the study (Table 1).

[0077]

Table I Clinical Measures

EDSS scores

	Pretreatment		Estriol Treatment		Post Treatment	
	3 mo.	6 mo.	9 mo.	12 mo.	15 mo.	18 mo.
6 RR	2.2	2.0	1.5	1.7	1.8	1.8
	(0.6)	(0.5)	(0.7)	(0.6)	(0.6)	(0.5)
4 SP	5.0	5.0	4.9	5.0	5.1	5.0
	(0.9)	(0.9)	(1.0)	(0.9)	(1.1)	(0.8)

9 Hole Peg Test scores

	Pretreatment		Estriol Treatment		Post Treatment	
	3 mo.	6 mo.	9 mo.	12 mo.	15 mo.	18 mo.
6 RR						
R	22.2	21.8	22.5	21.5	21.0	21.4
	(2.4)	(1.6)	(2.3)	(1.9)	(1.7)	(2.4)
L	24.8	22.9	24.3	23.3	23.0	22.7
	(3.2)	(1.6)	(2.5)	(2.1)	(2.1)	(2.3)
4 SP						
R	26.8	29.9	30.2	31.7	29.4	34.0
	(0.4)	(2.4)	(1.4)	(4.8)	(5.2)	(8.7)
L	23.5	25.6	22.7	24.8	26.7	25.0
	(1.4)	(2.5)	(1.7)	(2.6)	(0.7)	(1.8)

[0078] Interestingly, PASAT cognitive testing scores were significantly improved in the RR group, but not in the SP group (Fig 4). This improvement in PASAT scores in RR patients by 14.0% during treatment as compared to baseline, reached statistical significance, $P = 0.04$. It is unlikely that this improvement was entirely due to a practice effect of repeated testing because of the long time interval between testing (9 months) and because alternate versions of the test were used in each patient. This beneficial effect of estriol treatment on PASAT scores of RR MS patients is consistent with previous reports describing a beneficial effect of estrogen replacement therapy in surgically menopausal women and high dose estrogen treatment in Alzheimer's disease. Sicotte, et al. *Treatment of Women with Multiple Sclerosis Using Pregnancy Hormone Estradiol: A Pilot Study*. *Neurology*, 56 (8 Supp. 3):A75, April 2001, and Sicotte, et al. *Treatment of Multiple Sclerosis with the Pregnancy Hormone Estradiol*, Submitted to *Neurology* 2002, are herein incorporated by reference in their entirety.

[0079] EXAMPLE 2

[0080] Progesterone in combination with estrogen treatments has been shown to protect against endometrial proliferation and cancer. Indeed, estrogen cannot be given for a lengthy period of time in an "unopposed" fashion in any woman with a uterus. Thus, seven of the 12 patients wanted to remain on estriol after completion of the 18 month study. These patients were then put back on 8 milligrams of estriol and 100 milligrams of progesterone per day. In an extension phase of the study which began after completion of the post treatment phase. This extension phase was 4 months in duration. Each of the seven patients had an MRI every month during the 4 month extension phase. Additionally, each of the seven patients was examined neurologically and had serologic studies done at the end of this phase. No known negative effects 100 milligrams of progesterone in combination therapy with 8 milligrams of estriol treatment were noted.

[0081] In closing, it is noted that specific illustrative embodiments of the invention have been disclosed hereinabove. However, it is to be understood that the invention is not limited to these specific embodiments.

[0082] Accordingly, the invention is not limited to the precise embodiments described in detail hereinabove. With respect to the claims, it is applicant's intention that the claims not be interpreted in accordance with the sixth paragraph of 35 U.S.C. § 112 unless the term "means" is used followed by a functional statement.

[0083] While the specification describes particular embodiments of the present invention, those of ordinary skill can devise variations of the present invention without departing from the inventive concept.

WE CLAIM:

1. A method of treating a human patient exhibiting clinical symptoms of an autoimmune disease comprising administering at least one primary agent being an estrogen or estrogen receptor active agent at a therapeutically effective dosage in an effective dosage form at a selected interval to ameliorate the symptoms of the autoimmune disease.
2. The method of claim 1 wherein the autoimmune disease is a Th-1 cell mediated disease.
3. The method of claim 2 wherein the Th-1 cell mediated disease is selected from the group including multiple sclerosis, rheumatoid arthritis, autoimmune thyroiditis or uveitis.
4. The method of claim 1 wherein the primary agent is selected from the group of estriol, estrone, 17β -estradiol, aromatizable testosterone, estrogen receptor α agonists, estrogen receptor β agonists, estrogen receptor α antagonists or estrogen receptor β antagonists.
5. The method of claim 4 wherein the estriol is nyestriol, estriol succinate or estriol sulfamate or estriol dihexanate.
6. The method of claim 1 wherein the primary agent is estriol and the therapeutically effective dosage is about 4 to about 16 milligrams each 24 hours.
7. The method of claim 1 wherein the primary agent is estriol and the therapeutically effective dosage is about 8 milligrams each 24 hours.
8. The method of claim 1 wherein the primary agent is estriol and treatment results in patient serum concentrations of estriol of about 2 to about 30 nanograms per milliliter.

9. The method of claim 1 wherein the primary agent is estradiol and treatment results in patient serum concentrations of estradiol of about 2 to about 35 nanograms per milliliter.

10. The method of claim 1 wherein the primary agent is estrone and treatment results in patient serum concentrations of estrone of about 2 to about 18 nanograms per milliliter.

11. The method of claim 1 wherein the treatment results in serum levels of estrogen are equivalent to those of a women in the mid second trimester through the end of the third trimester of pregnancy.

12. The method of claim 1, further comprising administering at least one secondary agent at a therapeutically effective dosage in an effective dosage form at a selected interval concurrently with the primary agent.

13. The method of claim 12, wherein the secondary agent is progesterone which is administered at a dose of about 100 to about 200 milligrams per day.

14. The method of claim 12, wherein the secondary agent is a glucocorticoid.

15. The method of claim 14, wherein glucocorticoid is prednisone or methyl prednisone.

16. The method of claim 15, wherein the prednisone is administered at a dose of about 5 to about 60 milligrams per day.

17. The method of claim 15, wherein the methyl prednisone is administered at a dose of about 1 to about 2 milligrams per day.

18. The method of claim 12, wherein the secondary agent is at least one compound selected from the group comprising β -interferon, glatiramer acetate copolymer-1, azathioprine, cyclophosphamide, methotrexate, mitoxantrone or cyclosporin A.

19. The method of claim 18 wherein the β -interferon is interferon-beta 1a or interferon-beta 1b.

20. A method of treating a patient exhibiting clinical symptoms of multiple sclerosis comprising administering 8 milligrams of estriol orally each 24 hours to ameliorate the disease.

21. The method of claim 20, further comprising administering at least one secondary agent at a therapeutically effective dosage in an effective dosage form at a selected interval.

22. A method of treating a patient exhibiting clinical symptoms of multiple sclerosis to ameliorate the disease comprising administering estriol at a dose of about 4 to about 16 milligrams of estriol each 24 hours; and progesterone at a dose of about 100 to about 200 milligrams per 24 hours.

23. A method of treating a patient exhibiting clinical symptoms of multiple sclerosis to ameliorate the disease comprising administering estriol at a dose of about 4 to about 16 milligrams of estriol each 24 hours; and a glucocorticoid.

24. A method of treating a patient exhibiting clinical symptoms of multiple sclerosis to ameliorate the disease comprising administering estriol at a dose of about 4 to about 16 milligrams of estriol each 24 hours; and interferon β -1a at a dose of about 0 mg to about 30 mcg each week.

25. A method of treating a patient exhibiting clinical symptoms of multiple sclerosis to ameliorate the disease comprising administering estriol at a dose of about 4 to about 16 milligrams of estriol each 24 hours; and interferon β -1b at a dose of about 0 mg to about 0.25 mg every other day.

26. A method of treating a patient exhibiting clinical symptoms of multiple sclerosis to ameliorate the disease comprising administering estriol at a dose of about 4 to about 16 milligrams of estriol each 24 hours; and glatiramer acetate copolymer-1 at a dose of about 0 mg to about 20 mg each 24 hours.

27. The method of claim 3 or 20 wherein the multiple sclerosis is a relapsing remitting form or secondary progressive form of multiple sclerosis.

28. The method of claim 1 or 20 wherein the patient is a male or female.

29. A method of treating a patient exhibiting clinically isolated syndrome as a precursor to multiple sclerosis, comprising administering at least one primary agent being an estrogen or estrogen receptor active agent at a therapeutically effective dosage in an effective dosage form at a selected interval to delay the onset of additional symptoms multiple sclerosis.

30. The method of claim 29 comprising administering 8 milligrams of estriol orally each 24 hours.

31. The method of claim 29, further comprising administering at least one secondary agent at a therapeutically effective dosage in an effective dosage form at a selected interval.

32. A kit for treating a patient having an autoimmune disease comprising:
at least at least one primary agent being an estrogen or estrogen receptor active agent at a therapeutically effective dosage in an effective dosage form; and
at least one secondary agent in an effective dosage form.

33. The kit of claim 32, wherein the primary agent is selected from the group of estriol, estrone, 17 β -estradiol, aromatizable testosterone, estrogen receptor α agonists, estrogen receptor β agonists, estrogen receptor α antagonists or estrogen receptor β antagonists.

34. The kit of claim 32 wherein the secondary agent is selected from the group comprising: progesterone, glucocorticoid, β -interferon, glatiramer acetate copolymer-1, azathioprine, cyclophosphamide, methotrexate, mitoxantrone or cyclosporin A.

35. The kit 32 wherein the autoimmune disease is a Th-1 cell mediated disease and wherein the disease is multiple sclerosis, rheumatoid arthritis, autoimmune thyroiditis or uveitis.

36. A kit for treating a patient for multiple sclerosis comprising estriol in a dosage of about 4 milligrams to about 16 milligrams to be administered orally each day and injectable interferon β -1a in a dosage of about 0 to about 30 mcg to be injected intramuscularly once weekly.

37. A kit for treating a patient for multiple sclerosis comprising estriol in a dosage of about 4 milligrams to about 16 milligrams to be administered orally each day and injectable interferon β -1b in a dosage of about 0 to about 0.25 mg to be injected subcutaneously every other day.

38. A kit for treating a patient for multiple sclerosis comprising estriol in a dosage of about 4 milligrams to about 16 milligrams to be administered orally each day and glatiramer acetate copolymer-1 at a dose of about 0 mg to about 20 mg to be administered subcutaneously each day.

39. A kit for treating a patient displaying clinically isolated syndromes as a precursor to multiple sclerosis comprising:

at least one primary agent being an estrogen or estrogen receptor active agent at a therapeutically effective dosage in an effective dosage form; and
at least one secondary agent in an effective dosage form.

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FIG. 1a

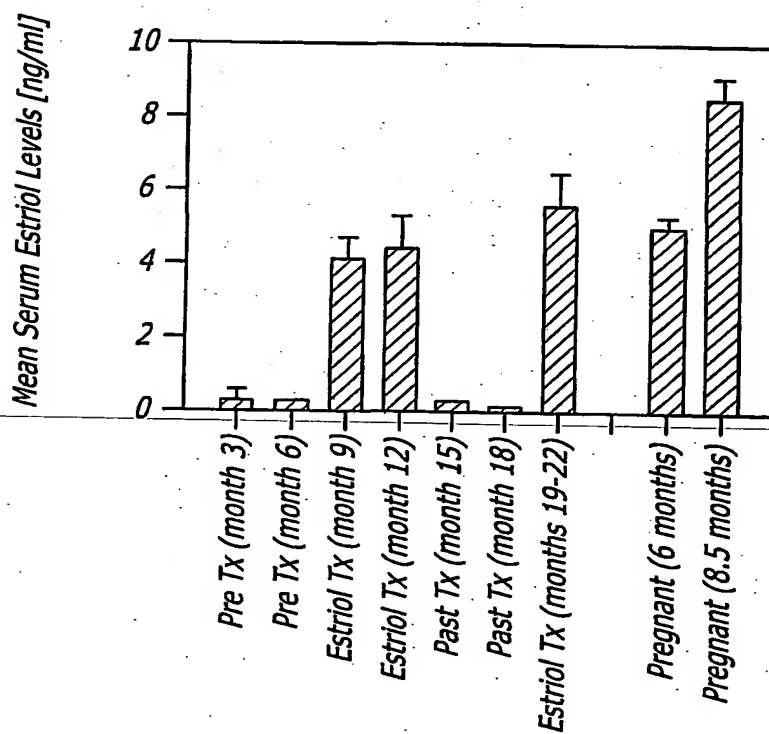
Pretreatment
I I I* I I I*
months 1-6

Estriol Treatment
I I I* I I I**
months 7-12

Post Treatment
I I I* I I I*
months 13-18

Estriol Treatment
I I I I*
months 19-22

FIG. 1b



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FIG. 2

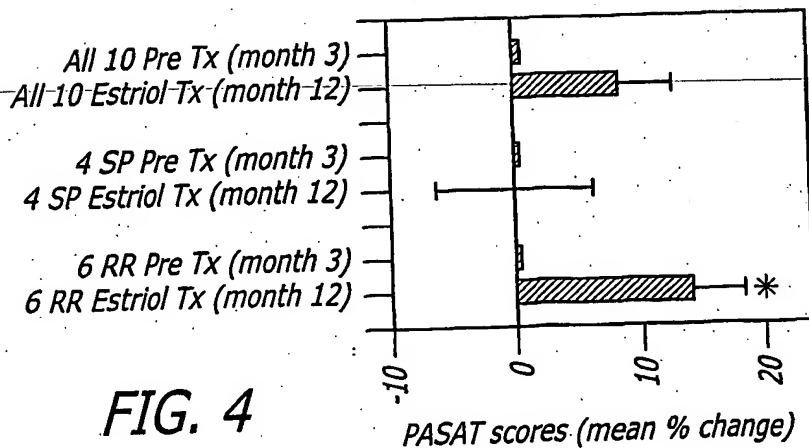
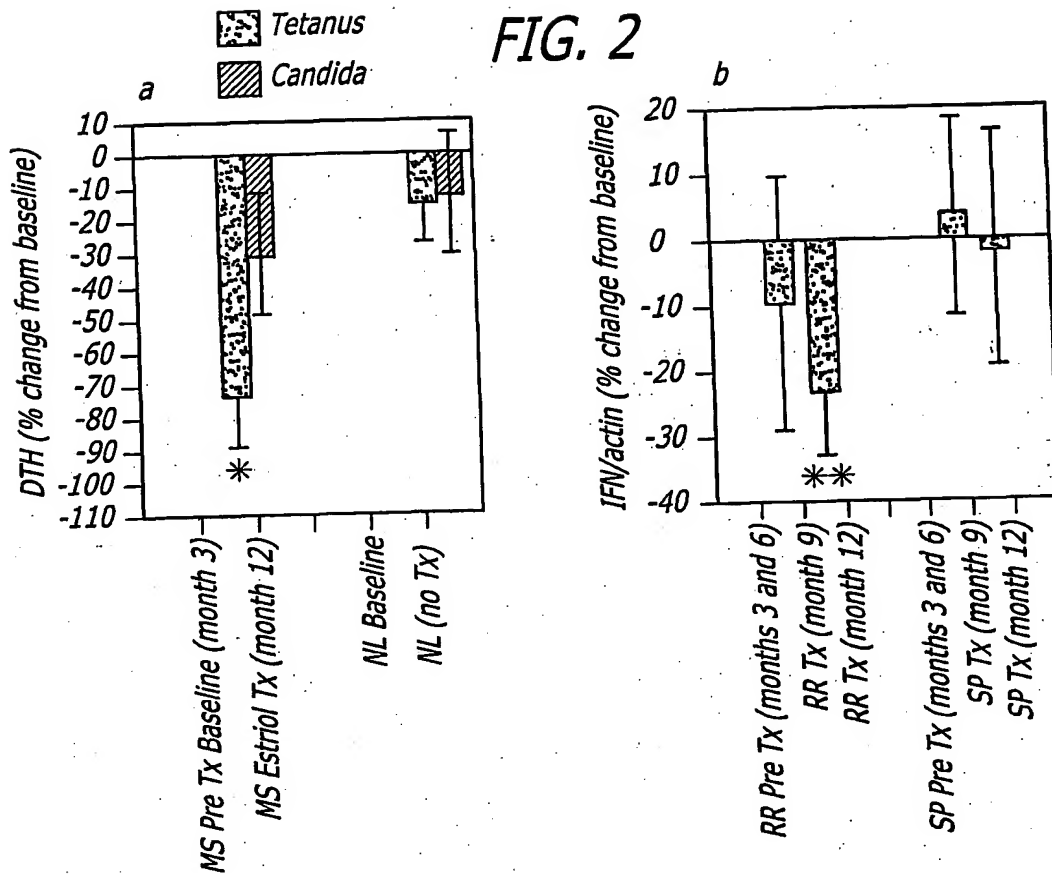
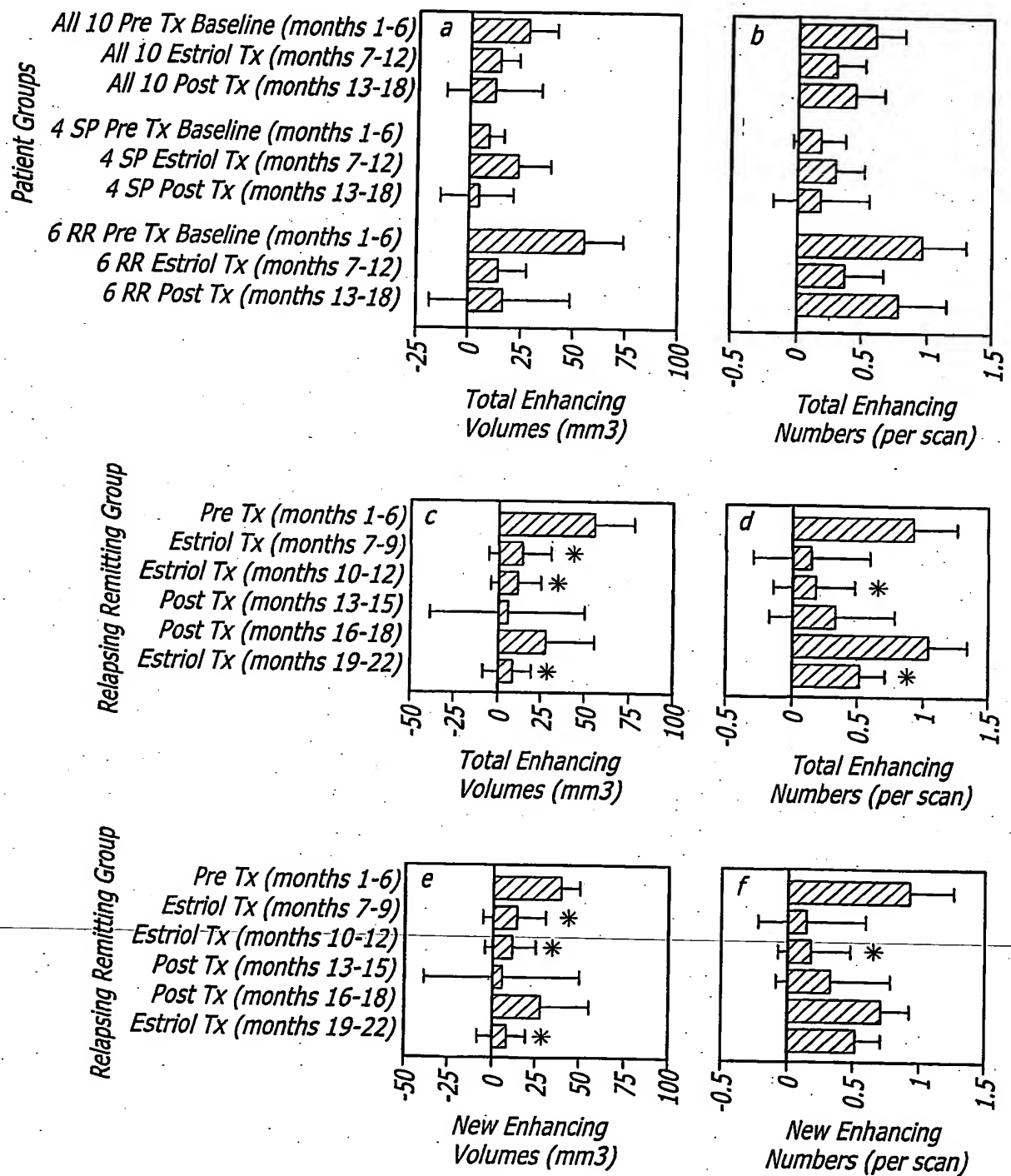


FIG. 4

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FIG. 3



INTERNATIONAL SEARCH REPORT

International application No.

PCT/US02/13407

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : A61K 31/56

US CL : 514/182

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/182

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 6,013,642 A (FOULKES ET AL) 11 January, 2000 (11/01/2000), see title, column 3, lines 56-67, claims 1-5.	1-39
Y	Database HCAPLUS on ACS. Accession No. 1999:288961, Kim et al. Estriol ameliorates autoimmune demyelinating disease. Implications for multiple sclerosis. Abstract. Neurology, 1999, Vol. 52, No. 6, pages 1230-1238, see abstract.	1-39

☐ Further documents are listed in the continuation of Box C.☐ See patent family annex.

* Special categories of cited documents:

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Date of the actual completion of the international search

15 July 2002 (15.07.2002)

Name and mailing address of the ISA/US
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